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ORIGINAL ARTICLE

Spectrophotometric analysis of Organophosphate degradation by *Pseudomonas* sp. strain BUR11

Santanu Pailan*
Assistant Professor, Department of Biotechnology, The University of Burdwan, Golapbag, Burdwan, Purba Barddhaman, West Bengal-713104
*Corresponding author's Email- spailan@biotech.buruniv.ac.in; santanu.pailan@gmail.com

ABSTRACT
A bacterial culture namely Pseudomonas sp. strain BUR11 was isolated from the agricultural soil sample of Burdwan. The bacterial strain capable of degrading parathion as sole source of carbon. Isolation, characterization, identification of the bacterial culture, details of parathion degradation study HPLC analysis followed by detection and identification of hydrolytic intermediates of parathion using GC-MS and LC-MS analysis were reported in my previous published research article. In the present article the author is going to report only spectrophotometric analysis of parathion degradation by the strain Pseudomonas sp. strain BUR11 for the first time. Before this no Organophosphate (OP) compounds degradation study was reported so far using spectrophotometric analysis. The present spectrophotometric analysis reported that 67.07% of residual parathion was utilized by the strain BUR 11 after 24 hr as sole source of carbon. This study also revealed that paranitrophenol was formed as hydrolytic intermediate of parathion during degradation study.
Keywords: Spectrophotometric analysis; Parathion; Microbial degradation; *Pseudomonas* sp. strain BUR11

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INTRODUCTION

Organophosphate (OP) insecticides are xenobiotic compounds which are esters of phosphoric acid and thiophosphoric acid. They are also called phosphotriesters due to presence of three phosphoester linkages [8]. Presently Organophosphates are most widely used insecticides [14]. Approximately, 2 million tons of pesticides are used per year throughout the world. The major consumers are Europe (45%) followed by USA (24%) and rest of the world (25%) [7]. These are applied not only on major crops like paddy, wheat, corn, mustard & cotton but also on fruits vegetables. The major reason of their popularity is due to their broad range of activity against insects and their low cost. Now a day OP compounds are also used as repellent of house hold insect-pests mites. These OP insecticides have a potential neurotoxicity to the non target organisms including human and other mammals. They act by inhibiting normal functions of cholinesterase, resulting in abnormal muscular activity, paralysis & death [1, 12]. Approximately 30, 00000 poisonings and 3, 00000 human deaths occur per year owing to OP ingestion and poisoning. OPs have been placed as highest priority chemicals by U.S. Environment Protection Agency (USEPA) and the tolerance level set by the Food and Drug Administration ranges between 1 to 8 µg ml^{-1} [4].Some hydrolysis product (*para*-nitrophenol, for example from parathion and 3, 5, 6 trichloro -2- pyridinol from chlorpyrifos) of these insecticides have higher half-life value and are persisted in the environment for long time [6, 13]. In the environment these toxic compounds enter the food chain, subsequently getting accumulated (bioaccumulation) and transformed at various tropic levels which has tremendous detrimental effect on ecosystem, disrupting normal ecological balance. Aquatic contamination of OP insecticides causes acute and chronic poisoning of fish and other organisms. An important way to avoid ecological damages and health problems to other organisms (including man) due to the presence of OP insecticides is to reduce their concentration in the environment, preventing either lixiviation to groundwater or possible incorporation to natural food chains [2, 3, 10].

Several methods of lowering environmental pollution include chemical treatment, incineration and physical removal of the contaminated soil. These are most expensive and inefficient methods. On the other hand, biological technique such as microbial degradation is more efficient, inexpensive, eco-friendly and achievable [5]. Most of these OP insecticides are degraded by soil micro flora like bacteria, fungi, algae and cyanobacteria [9, 10]. Majority of these OP compounds are degraded by microorganisms in the environment as source of phosphorus or carbon or both. In the present study author revealed a spectrophotometric analysis of parathion degradation by the strain *Pseudomonas* sp. BUR11 for the first time. The details such as isolation, characterization, phylogenetic position based of 16S rRNA gene based molecular phylogenetic approach and parathion degradation studies by HPLC method by this bacterial strain was elaborated in Pailan and Saha, 2015 [11]. By spectrophotometric analysis, the strain BUR11 degrades 67.07% of parathion within 24 hr. UV-Vis scanning analyses also revealed the presence of paranitrophenol in the degradation samples indicating that the bacterial strain degrades parathion through the production of paranitrophenol.

MATERIAL AND METHODS

Chemicals:

Parathion and its degradation intermediates were purchased from Sigma. Solvents used in this study were of HPLC grade. Microbiological media, components, general chemicals were purchased from HiMedia, India as mentioned in Pailan and Saha, 2015 [11].

Isolation, Characterization and Identification and Growth of the isolate BUR11

Soil sample collection, enrichment technique, medium used for isolation, cultivation and further degradation studies of parathion, phenotypic characterization, identification of the bacterial isolates BUR11 on the basis of 16S rRNA gene based molecular phylogenetic approach and growth pattern in presence of parathion were discussed elaborately in Pailan and Saha, 2015 [11].

Degradation studies of parathion by strain BUR11

During biodegradation studies, the BUR11 strain was initially pre-cultured in Triptic soya broth (TSB) to increase the bacterial cell density, harvested by centrifugation at 10,000 rpm for 10 min, washed three times with sterilized normal saline. 200 µl of cell suspension having OD value 0.5 at 600 nm inoculated into 50 ml of Mineral Medium (in Erlenmeyer flask) supplemented with 200 ppm of parathion as sole carbon source. Control flaks were remained un-inoculated. The incubation was done at 37^0C on a shaker at 120 rpm for 4 days.

After a regular 24h time interval entire portions of broth culture was withdrawn aseptically, centrifuged at 10,000 rpm for 10 min. Supernatant was taken in a fresh micro centrifuge tube for extraction procedure. Culture Supernatant was extracted twice with equal volume of ethyl acetate (HPLC grade) followed by dehydration with anhydrous sodium sulfate. The extracted sample was concentrated by performing rotary evaporator. One milliliter of Acetonitrile (HPLC grade) was added to the extracted sample, mixed it properly and filtered it through 0.22 µm filter (Millipore filter). The sample was used to determine parathion degradation spectrophotometrically.

Spectrophotometric Analysis of parathion degradation

Parathion degradation by BUR11 was analyzed using UV-Visible spectrophotometer (Varian, Cary 50 Bio UV- Visible spectrophotometer) at 274 nm. A UV-Visible scanning of the sample of parathion degradation was also carried out at 200 nm to 800 nm. Quantitative analysis of residual as well as degraded parathion was carried out by taking OD at 274 nm. A standard curve of analytical parathion was also prepared of by taking OD at 274 nm. For detection and identification of paranitrophenol the OD value was taken at the range of 380 nm. The λ_{max} of the parathion and paranitrophenol was fluctuating or deviating little bit during UV-Visible scanning due to extraction of the sample or any other unkown noise present in the sample coming from broth medium.

RESULT AND DISCUSSION

Spectrophotometric analysis of parathion degradation

After four days of parathion degradation studies by strain BUR11 by Spectrophotometric analysis, 67.07% (Figure 1) of parathion was degraded. HPLC analysis of the parathion degradation by the same strain was 62 % (Pailan and Saha, 2015-[11]). This may be due to the efficiency and the different detection level of the OP compound by the different analytical instruments. In the present study the amount of parathion degraded, after every 24 hr interval and percent of degradation elaborately shown in Figure 2 respectively. After first 24 hr 86 µg of parathion was utilized by the strain *Pseudomonas* sp. BUR11 followed by 20 µg, 16.8 µg and 9.2 µg respectively for next every 24 hr (Figure 2). Scanning spectra under UV-Visible (Figure 3) identifies paranitrophenol was generated as major hydrolysis

intermediate of parathion during degradation present study. The pattern of scanning spectra also reveals that the residual parathion in the flask was gradually depleted by the utilization of BUR11 by formation of paranitrophenol as time passage.

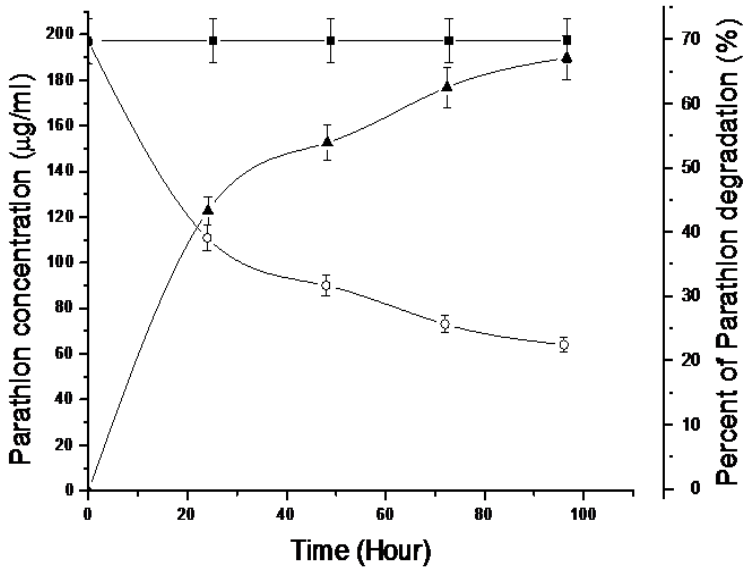

Figure 1. Spectrophometric parathion degradation profile of BUR11. '■' indicates the control line of parathion; 'O' indicates the amount of parathion degraded after specific time interval and '◇' indicates the percent of parathion degradation with passage of time.

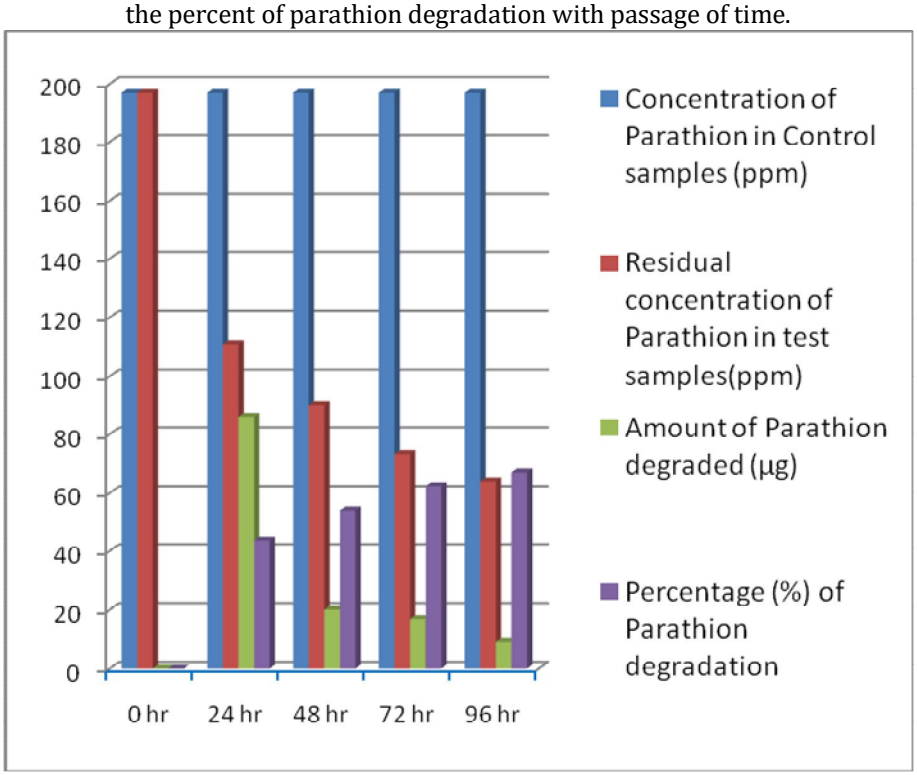

Figure 2. Bar chart represents the residual parathion, amount of parathion degraded and percent (%) of degradation in every 24 hr interval.

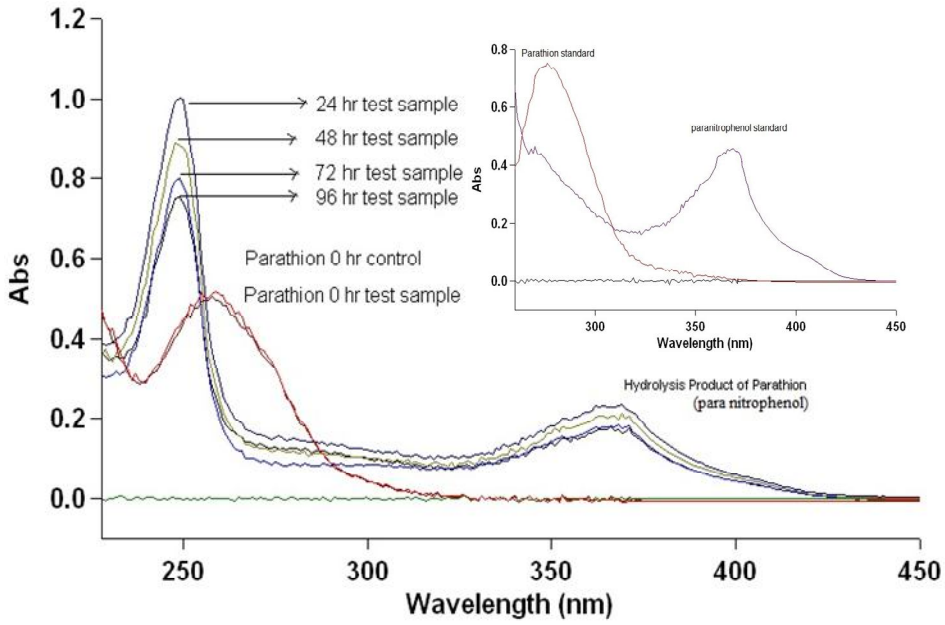

Figure 3. UV-Vis scanning of the test sample of parathion degradation by the bacterial strain BUR11. In the inset of the this pictorial diagram two curves, one indicating the standard curve of parathion (λ_{max}=274 nm; approximately) and the second line representing the standard of paranitrophenol (λ_{max}=380 nm; approximately). In the parathion degrading test samples (24 hr interval), the concentrations of parathion (proportional to the OD value) were decreasing gradually where simultaneously paranitrophenol as major hydrolytic intermediate was detected (λ_{max}=380 nm; approximately).

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